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Description

Field of the Invention

The invention relates to a method of determining levels of extrinsic and intrinsic clotting factors and protein C based upon the reaction rate of the observed clot formation and the first derivative of the reaction rate of observed clot formation.

Background of the Invention

Clotting of blood is a complicated process involving a large number of blood components including fibrinogen and prothrombin which is converted to thrombin. It has long been recognized that many aspects of unexplained bleeding or abnormal clotting can be explained in terms of improper levels of these materials in the blood. For instance, states of hypo-fibrinogenemia or hyper-fibrinogenemia may result from hepatic disease, from disseminated intravascular coagulation, from fibrinolytic syndrome, neoplastic disease, and postoperatively due to trauma. By monitoring the fibrinogen, thrombin and prothrombin levels within the blood, a physician may acquire meaningful data concerning the patient's blood clotting abilities. For example, the Activated Partial Thromboplastin Time (APTT) Test measures coagulation factors of the intrinsic pathway. These factors include Factors XII, XI, IX, VIII, X, V, II and I which may be abnormal based on heredity or heparin therapy. Thus, the APTT test is useful as a presurgical screen and for monitoring heparin therapy. Similarly, fibrinogen testing (by the Thrombin Time (TT) test or quantitative fibrinogen test) provides useful diagnostic data when unexplained bleeding or abnormal clotting occurs.

Within the past decade, evidence has accumulated linking soluble clotting factors with ischemic heart disease. Studies have shown that elevated blood plasma concentrations of Factors VIIa, VIIIc, and fibrinogen appear to be associated with an increased risk of heart attack and cardiovascular death. Patients who are in such a hypercoagulable state require careful monitoring. In the past, decisions to change medication, adjust dosage, or proceed with surgery were made based upon clotting time.

The process of blood coagulation occurs as a series of complex steps which terminate in the formation of a fibrin clot. Clot formation may occur by activation of the intrinsic pathway. In this system, coagulation factors circulate in the form of inactive precursors which are converted into an active form, which in turn activates the next clotting factor in sequence, i.e., proenzyme Factor XII is converted to its enzyme XIIa which in turn converts the zymogen Factor XI to the enzyme Factor XIa, which then activates Factor IX, in the presence of calcium. The enzyme Factor IXa in the presence of Factor VIII and phospholipid activates Factor X. This reaction is greatly increased by the prior exposure of Factor VIII to thrombin or Factor Xa.

In the extrinsic pathway, Factor X can be activated by either a complex of thromboplastin and Factor VII, or a complex of platelet phospholipid activated Factor IX and Factor VIII. Activated Factor X, in the presence of calcium, Factor V and platelet phospholipid activates Factor II (prothrombin) which is cleaved to form thrombin which converts Factor I (fibrinogen) to fibrin in blood plasma.

The process of blood coagulation is modified by a number of positive and negative feed back loops and by interaction between these pathways. For example, thrombin and Factor Xa, formed either by activation of the intrinsic or extrinsic pathway, feed back to activate Factor VIII and Factor V. Factor Xa feeds back to initially increase and then to inhibit its own activation by Factor VIIa. The intrinsic and extrinsic pathways are also linked. For example, Factor VII is activated by Factor IXa, XIIa and XIa and Factor VIIa can activate Factor IX.

The basis of in vitro coagulation testing has been the determination of the increase in turbidity or viscosity of a sample, caused by the conversion of fibrinogen to fibrin during clot formation. The screening tests for coagulation disorders include the prothrombin time (PT) and the activated partial thromboplastin time (APTT). Essentially, the screening tests for coagulation disorders are designed to detect a significant abnormality in one or more of the clotting factors and to localize this abnormality to various steps in the coagulation pathway. For example, APTT measures coagulation factors of the intrinsic pathway, including Factors XII, XI, IX, VIII, X, V, II and I which may be abnormal based on heredity or heparin therapy. APTT is therefore useful as a presurgical screen and for monitoring heparin therapy.

The APTT is performed by adding an activator such as kaolin, ellagic acid, or silica, for example, with phospholipid to plasma. This activates Factors XII and XI. Phospholipid substitutes for platelet in the activation of Factor VIII by Factors IX, VIII and V. Blood coagulation is initiated in this clotting test by adding calcium. Factor VII is the only factor not affected by the partial thromboplastin time and the APTT is, therefore, normal in patients with a Factor VII deficiency.

The prothrombin time (PT) test is performed by adding tissue thromboplastin with calcium to plasma. This initiates clotting by activating Factor VII which in turn activates Factor X which in the presence of Factor V,

converts prothrombin to thrombin and the thrombin which is so produced converts fibrinogen to fibrin. PT therefore bypasses the intrinsic clotting pathway and is normal in patients with deficiencies of Factors XII, XI, IX and VIII. PT is abnormal in patients with deficiencies of Factors VII, X, V, prothrombin or fibrinogen.

As a result, substantial efforts have been made to measure these clotting components. Most methodologies rely upon immunologic and clotting techniques although clearly the latter is preferred. The immunologic techniques, although generally capable of precisely defining the levels of the various components within the blood stream, are incapable of distinguishing between active and inactive forms. Accordingly, the immunologic methods are felt to be less accurate with respect to the patient's actual clotting ability. Consequently, the results obtained by clotting techniques are preferred as being more clinically significant.

The human eye was the first clot detection system used for coagulation testing, e.g., a normal sample produces a strong gel clot; samples producing thin,watery, webby-type clots are indicative of some coagulation abnormality. Automated coagulation instrumentation, both mechanical and optical density-based, provide data about the end point of the clotting times in the various coagulation tests, e.g., PT and APTT. Typically, most instruments detect the formation of a clot by monitoring either optical turbidity or electrical conductivity. The latter represents the traditional approach employed by the so-called fibrometer-type of instrument. Effectively, this instrument measures increasing conductivity which may be correlated to the formation of clots. Similarly, turbidity may be optically sensed by the decrease in light transmission due to the formation of a clot. Certainly with the normal PT or APTT tests, these methods have found widespread acceptance despite the fact that each test has associated therewith a level of indefiniteness regarding the point at which the clot is determined to have occurred.

A more advanced instrument, such as the KoaguLab® (Ortho Diagnostic Systems Inc., Raritan, New Jersey) generates a printed graph of the clotting reaction. Clinicians can tell by the shape of the curve generated whether or not the clotting time is reliable, thus providing a stronger information base for their therapeutic decisions. A graph which plots turbidity against reaction time is referred to as "clot signature". As used in the Koagulab® system, the total turbidity change attained at the maximum clotting endpoint is "delta", the reaction rate of the observed clot formation is Velocity, and the derivative of Velocity or the maximum acceleration of observed clot formation is Acceleration. KoaguLab® may be used to perform PT and APTT assays. These are performed be adding brain thromboplastin or activated partial thromboplastin and calcium chloride respectively, to a plasma sample and determining the time at which the clot forms. Reagents useful for these purposes include, for instance, Ortho Quantitative Fibrinogen Assay (Q.F.A.), Ortho Q.F.A. Thrombin (Human), Ortho Q.F.A. Buffer, Ortho Activated PTT Reagent, Ortho Activated Thrombofax™ Reagent, Ortho Brain Thromboplastin, Fibrindex™ Thrombin, Ortho Plasma Coagulation Controls (obtainable from Ortho Diagnostic Systems Inc., Raritan, New Jersey). Either of the two generalized embodiments of the methods of the present invention may be utilized for calculating PT or APTT coagulation times.

The clot signature essentially adds a qualitative fibrinogen measurement to the standard PT and APTT tests, which may prove useful in detecting certain disease states, including hypercoagulability.

EP-A-0 014 039 relates specifically to the measurement of the rate of thrombin development. The method relies on a measurement of the amount of thrombin produced, preferably by use of a spectrophotometric assay using a chromogenic substrate. Although other methods may be used it is not suggested that a clotting time should be measured.

Moreover, the method disclosed in said document does not involve the production of profiles of the first or second derivatives of a graph of degree of clotting against time.

The whole basis of the disclosure is the use of a graph plotting the logarithm of thrombin production (as represented by an increase in optical density) against time. It is the first derivative of this graph which is used.

DE-A-3 734 889 relates to a particular method of determining the amount of Protein C in a sample.

The result of the assay is determined by observing a time or an optical density. There is no disclosure or suggestion that any derivative function should be used.

Summary of the Invention

The present invention relates to a method for determining the level of an extrinsic or an intrinsic clotting factor or protein C comprising:

(A)

(i) carrying out a clotting time test on a normal plasma sample,

(ii) measuring the degree of clot formation as a function of time for the normal plasma sample, and

(iii) deriving either the first or the second derivative of the function measured in step (ii);

(B)

(i) carrying out the same clotting time test on a test plasma sample,

(ii) measuring the degree of clot formation as a function of time for the test plasma sample, and

(iii) deriving either the first or the second derivative of the function measured in step (ii); and

(C) comparing either the first or the second derivative derived in step A (iii) with the corresponding derivative derived in step B (iii).

Also provided is a method for identifying abnormal extrinsic or intrinsic clotting factor or Protein C levels comprising:

(i) carrying out a clotting time test on a test sample;

(ii) measuring the degree of clot formation as a function of time for the test sample;

(iii) deriving both the first and the second derivatives of the function measured in step (ii);

(iv) determining the level of said clotting factor or Protein C from a correlation of said first and second derivatives.

In the present invention, it was surprisingly found that Velocity (the reaction rate of the observed clot formation), and Acceleration (the derivative of Velocity or the maximum acceleration of observed clot formation) are useful indicators for quantitating the various clotting factors and protein C. The "clot signature" (turbidity versus reaction time) therefore becomes a useful clinical tool for monitoring drug therapy (e.g., heparin or coumarin) and provides useful information concerning diagnosis and treatment of disorders such as liver disease, hemophilia, protein C deficiency or DIC.

Accordingly, the present invention provides a method of determining levels of extrinsic and intrinsic clotting factors and protein C. This and further objects of the present invention will become apparent from the following more detailed description.

Description of the Drawings

Figure 1 is a graph showing the correlation of Factor II levels with Factor II Velocity and acceleration.

Figure 2 is a graph showing the correlation of Factor VII level to Factor VII Velocity and Acceleration.

Figure 3 is a graph showing the correlation of Factor X concentration with Factor X Velocity and Acceleration.

Figure 4 is a graph showing the correlation of protein C concentration with protein C Velocity and Acceleration.

Figure 5 is the clot signature information for four patients with liver disease. The "clot signature" is a graphical depiction of turbidity versus time (seconds) as generated by a KoaguLab® device. The left hand signature for each patient indicates the results of a PT assay. The right hand signature, an APTT assay.

Figure 6 is the clot signature information for a hemophilia A patient's sample, Factor VIII less than 1%, generated by a KoaguLab® device, depicting turbidity versus time (seconds) for a PT (left hand signature) and APTT (right hand signature) assay.

Figure 7 is the clot signature information from a protein C deficient plasma, generated by a KoaguLab® device, depicting turbidity versus time for a PT (left hand signature) and APTT (right hand signature) assay.

Figure 8 is the clot signature information for three DIC samples generated by a KoaguLab® device, depicting turbidity versus time for a PT (left hand signature) assay for each of three patients.

Detailed Description of the Invention

The present invention provides a method to determine levels of extrinsic and intrinsic clotting factors and protein C using PT or APTT factor-based assays and the observed rate of clot formation (Velocity) and the first derivative of the observed rate of clot formation (Acceleration) determined in test samples and compared with normal plasma samples. The comparisons can be made in several ways. The Velocity or Acceleration value in the test sample can be compared directly with the Velocity or Acceleration value in a normal plasma sample or an individual factor level can be correlated with that factor's Velocity and Acceleration in a test sample, which is compared with known, normal ranges.

A prothrombin time test (PT) is used to determine deficiencies of clotting factor activity in the extrinsic pathway. By adding tissue thromboplastin, e.g., ORTHO Brain Thromboplastin, to normal anticoagulated plasma, the clotting mechanism is initiated. The endpoint of the assay procedure is the appearance of a solid gel clot, which may be determined visually, mechanically, or by optical means. If there is a deficiency of factor activity in the extrinsic pathway, the time required for clot formation will be prolonged beyond that expected for normal plasma. Clotting times on patient plasma samples are compared with normal plasma samples. In this invention, the reaction rate of th observed rat of clot formation (Velocity) and the first derivative of the observed rate of clot formation (Acceleration) in a factor-based PT assay are determined in test samples and compared with normal plasma samples.

An activated partial thromboplastin time test (APTT) is used to indicate abnormalities in most of the procoagulant clotting factors. The APTT assay is a useful sensitive procedure for generating heparin response curves and for screening deficiencies of clotting factors in the intrinsic pathway. In this invention, activated THROMBOFAX (manufactured by ORTHO Diagnostic Systems Inc., Raritan, New Jersey), a buffered reagent which contains phospholipids and a plasma activator, is mixed with test plasma and a specific intrinsic factor deficient plasma. The clotting reaction is initiated by the addition of ionic calcium and the test result is the time required for clot formation.

In one embodiment of this invention, the reaction rate of the observed clot formation, or Velocity, in a factor-based PT or APTT assay is determined for both test and normal plasma samples and the Velocity values compared. This method involves determining the reaction rate of the observed clot formation in a normal plasma sample, determining the reaction rate of the observed clot formation in a test plasma sample, and then comparing the reaction rate determined in the test sample with the reaction rate determined in a normal plasma sample.

In another embodiment of this invention, the first derivative of the reaction rate of the observed clot formation, or Acceleration, in a factor-based PT or APTT assay is determined for both test and normal plasma samples and the values compared. The method involves determining the first derivative of the reaction rate of the observed clot formation in a normal plasma sample, determining the first derivative of the reaction rate of the observed clot formation in a test plasma sample, and comparing the first derivative determined in the test sample with the derivative determined in the test sample with the derivative determined in a normal plasma sample.

The methods of the invention can be used to determine levels of extrinsic and intrinsic clotting factors and protein C. It is also useful for determining levels of extrinsic clotting Factors II, VII and X for monitoring coumarin therapy. In an embodiment of this invention, levels of extrinsic clotting Factors II, VII, and X for monitoring coumarin therapy is provided. A factor-based PT assay is performed to measure said extrinsic clotting factors. The Velocity is determined and compared with the reaction rate observed in normal plasma samples. In another embodiment, the first derivative of the observed clot formation is determined for both test and normal plasma samples, and the results compared.

The method is also useful for determining levels of intrinsic clotting Factors XII, XI, IX, VIII, V, X and II for monitoring heparin therapy. In another embodiment, a method of determining levels of intrinsic clotting Factors XII, XI, IX, VIII, V, X, and II is provided. A factor-based APTT assay is performed and the reaction rate of observed clot formation is determined and compared with the reaction rate observed in normal plasma samples. In another embodiment, the Acceleration is determined for both test and normal plasma samples, and the results compared.

Individual clotting factors can also be determined in accordance with this method. Individual intrinsic or extrinsic clotting factors are determined by performing a PT or APTT assay using a specific factor deficient plasma, determining the reaction rate of observed clot formation in a normal plasma sample mixed with said factor deficient plasma, determining the observed rate of observed clot formation in a test sample mixed with said factor deficient plasma, and comparing the Velocity values obtain. In another embodiment, the first derivative of the reaction rate of observed clot formation is determined for a test sample and compared with the value for a normal plasma sample, where both samples are mixed with the specific factor deficient plasma.

Protein C levels are also determined in accordance with the teachings of this method by performing a modified APTT assay by activating the protein C with a protein C activator, such as snake venom. The reaction rate of the observed clot formation in a test sample mixed with protein C deficient plasma is compared with the Velocity determined in a normal plasma sample. The prolongation of the clotting time is proportional to the amount of protein C in the test plasma sample.

Abnormal clotting factors and protein C are identified by the method of this invention by determining the Velocity and Acceleration values for a test sample and normal plasma and then correlating the level of the clotting factor with Velocity and Acceleration. An example of the correlation of Factor II levels with Factor II Velocity and Acceleration, according to this method is shown in Figure 1. Figure 2 depicts an example of the correlation of Factor VII levels to Factor VII Velocity and Acceleration. In Figure 3, a correlation of Factor X concentration with Factor X Velocity and Acceleration is shown. Figure 4 depicts an example of the correlation of protein C concentration with protein C Velocity and Acceleration in accordance with this method.

The invention is further illustrated by means of the following xamples. These examples ar meant to be illustrations only and are not intended to limit the present invention to the specific embodiments.

Exampl 1

PT and APTT Cl t Signature Data For 20 Normal Volunteers

The ranges for clot signature data obtained in PT and APTT assays with fresh normal patient samples are listed below:

	PT	APTT
Clotting Time	11.7-13.9	30.1-35.5
Delta	1867-3539	2919-3644
Velocity	503-1092	299-553
Acceleration	404-861	115-301

Example 2

PT Clot Signature For Patients Undergoing Coumarin Oral Anticoagulant Therapy

The clot signature therapy for four patients undergoing coumarin therapy were:

	PT
Clotting Time	15.3-24.6
Delta	1841-3548
Velocity	245-727
Acceleration	101-397

When these clot signatures are compared with those of normal PTs, the steepness of the curve is lower as reflected in the velocity and acceleration values. The delta values for the coumarin samples are about the same as those found with normal plasma, which is expected because the delta value correlates with fibrinogen, which is not affected by coumarin. The clotting Factors II, VII and X are affected by coumarin and consequently, the rate of clot formation is more reflective of the different levels of the clotting factors. The velocity range found with normal patient samples was 503 to 1092. The velocity values with coumarin samples ranged from 245 to 727, which is low normal to below normal. The clot signature could therefore be useful to indicate whether a patient was receiving too much or too little coumarin to ensure that clot formation would be lessened.

Example 3

APTT Clot Signature Data In In Vitro Heparinized Patient Samples

The clot signature data for three patients undergoing heparin anticoagulant therapy were:

	APTT
Clotting	45.6-104
Delta	1185-3711
Velocity	142-514
Acceleration	11-186

The clot signature in this case is a useful tool to alert the lab immediately when a longer maximum end point is required. Many patients on heparin therapy have higher than normal fibrinogen levels. For this reason, the delta value is a better indicator of fibrinogen levels.

Low velocity values for these patients may indicate a reduced reaction rate of clot formation indicating that a patient may be receiving too high a dose of heparin and could experience clinical bleeding, especially if additional heparin is administered. On the other hand, a high velocity value indicates a rapid rate of clot formation. A patient such as this requires additional heparin or may be reflecting a resistance to heparin. If administration of additional heparin reduces the patient's velocity value, the clinician would be able to monitor the patient by balancing the APTT clotting time with the velocity value.

Example 4

PT/APTT Clot Signatures for Patients with Confirmed Liver Disease

Figure 5 compares the PT and APTT clot signature information for four patients with liver disease. The four patients are obviously quite different just from a cursory glance at the clot signatures. The delta values indicate that patient 3 had a normal fibrinogen level, whereas patients 1 and 2 have low fibrinogen levels. Patient 4 is probably at a low normal fibrinogen level. From the PT Velocity values, patients 1 and 2 appear to have low concentrations of one or more of the Factors V, X, VII and II, whereas patients 3 and 4 appear to have low normal levels. From the APTT Velocity values, patients 1 and 2 appear to have low levels of one or more Factors XII, XI, V, VIII, II, IX and X levels, whereas patients 3 and 4 exhibit normal levels. The prolonged APTT clotting times for patients 3 and 4 were due to heparin rather than abnormally low levels of factors, since the APTT Velocity values do not indicate abnormally low clotting factors. The analyses provided for these four patients with confirmed liver disease were as follows:

Patient	F II	F VIII	FIX	FX	AT III
1	36%	914%	--	38%	28%
2	28%	702%	36%	47%	38%
3	47%	280%	33%	76%	30%
4	58%	926%	43%	66%	30%

Example 5

PT/APTT Clot Signatures for Patient with Hemophilia (Factor VIII less than 1%)

The PT and APTT clot signatures for a hemophilia A patient's sample, Factor VIII less than 1%, is illustrated in Figure 6. The PT clot signature appears to be normal. The Velocity and Acceleration values suggest that the prolonged APTT time was caused by a factor deficiency or an inhibitor. The clinical history of the patient would assist the laboratory in selecting what tests should b performed next to identify the cause of the prolonged APTT clotting tim .

Example 6

PT/APTT Clot Signatures for a Patient with Protein C Deficiency

The PT and APTT clot signature information from a Protein C deficient patient does not appear to differ from that obtained with a normal plasma as shown in Figure 7. One would not anticipate that the Protein C deficiency sample would have abnormal PT and APTT clotting reactions, unless the patient experienced some external trauma.

Example 7

PT/APTT Clot Signatures for Three Patients with DIC

The last example presented is the clot signatures for three DIC samples as shown in Figure 8. The clot signatures suggest that patients 21 and 22 have low fibrinogen levels as indicated by the PT Delta values (left hand signature). Patient 23 appears to have a low normal fibrinogen level from the PT Delta value. The rate of clot formation as indicated by Velocity values in both the PT and APTT clot signature data for patients 21 and 22 indicated significantly lower than normal Velocity value. This suggests that these two patients are in a critical state in contrast to patient 23. Patient 23 is either on the road to recovery or just about to enter a crisis. The significance of the clot signature is just beginning to emerge, but already there are indications that it can assist the clinical laboratory to gain valuable insight into a fuller clinical understanding of the patient's hemostatic state.

As seen from the above, a simple reproducible method for determining levels of extrinsic and intrinsic clotting factors and protein C is provided.

Claims

1. A method for determining the level of an extrinsic or an intrinsic clotting factor or protein C comprising:
   (A)
   (i) carrying out a clotting time test on a normal plasma sample,
   (ii) measuring the degree of clot formation as a function of time for the normal plasma sample, and
   (iii) deriving either the first or the second derivative of the function measured in step (ii);
   (B)
   (i) carrying out the same clotting time test on a test plasma sample,
   (ii) measuring the degree of clot formation as a function of time for the test plasma sample, and
   (iii) deriving either the first or the second derivative of the function measured in step (ii); and
   (C) comparing either the first or the second derivative derived in step A (iii) with the corresponding derivative derived in step B (iii).

2. A method for identifying abnormal extrinsic or intrinsic clotting factor or Protein C levels comprising:
   (i) carrying out a clotting time test on a test sample;
   (ii) measuring the degree of clot formation as a function of time for the test sample;
   (iii) deriving both the first and the second derivatives of the function measured in step (ii);
   (iv) determining the level of said clotting factor or Protein C from a correlation of said first and second derivatives.

3. The method of claim 2, wherein the level of said clotting factor or Protein C is expressed as a percentage of said clotting factor or Protein C.

4. The method of claim 2 or claim 3, wherein said determining step (iv) comprises plotting said level against said first and second derivatives.

5. The method of any one of claims 2 to 5, wherein the results of the determining step are provided as a graphical representation.

6. The method of claim 4 or claim 5, wherein the abnormal level is determined from analysis of the plot or graphical representation.

7. The method of claim 6, further comprising the step of selecting at least one drug dose for a patient or one clinical test to be performed on the patient based on the result of the analysis.

8. The method of any one of claims 1 to 7, wherein the clotting time test is a prothrombin time test and the method is for identifying extrinsic clotting factor levels.

9. The method of any one of claims 1 to 7, wherein the clotting time test is an activated partial thromboplastin time test and the method is for identifying intrinsic clotting factor levels.

10. The method of claim 8, which is used for determining relative levels of extrinsic clotting factors II, VII and X for monitoring coumarin therapy.

11. The method of claim 9, which is used for determining functional levels of intrinsic clotting factors XII, XI, IX, VIII, V, X and II for monitoring heparin therapy.

12. The method of any one of claims 8 to 11, wherein said clotting time test is performed using plasma deficient in a specific clotting factor to be determined mixed with either a normal plasma sample or a test sample.

13. The method of any one of claims 1 to 7, wherein the clotting time test is a modified activated partial thromboplastin time test and the method is for identifying abnormal protein C levels.

14. The method of claim 13, wherein said clotting time test is performed using plasma deficient in Protein C mixed with either a normal plasma sample or a test sample.

15. The method of claim 14, wherein the clotting time test is performed by activating the Protein C with a Protein C. activator.

16. The method of claim 15, wherein the Protein C activator is snake venom.

17. The method of any one of claims 1 to 16, wherein the degree of clot formation is measured by observing the degree of turbidity in the sample.

18. The method of claim 17, further including the steps of:
determining the value of the total turbidity change at the maximum clotting end point in the normal plasma sample;
determining the value of the total turbidity change as the maximum clotting end point in the test plasma sample; and
comparing said normal sample value with said test sample value.

19. The method of claim 18, in which said test sample value is used to indicate fibrinogen levels.

Patentansprüche

1. Verfahren zur Bestimmung des Spiegels eines extrinsischen oder eines intrinsischen Gerinnungsfaktors oder von Protein C, umfassend:
(A)
(i) Das Durchführen eines Gerinnungszeittests an einer normalen Plasmaprobe,
(ii) Messen des Grades der Gerinnselbildung als Funktion der Zeit für die normale Plasmaprobe, und
(iii) Bilden von entweder der ersten oder der zw iten Abl itung der in Schritt (ii) gemessenen Funktion;
(B)
(i) Durchführen desselben Gerinnungszeittests an einer Plasmat stprobe,
(ii) Mess n des Grades der Gerinns lbildung als Funktion der Zeit für die Plasmatestprobe und
(iii) Bilden entweder der ersten oder der zweiten Ableitung der in Schritt (ii) gemessenen Funktion; und

(C) Vergleichen von entweder der ersten oder der zweiten Ableitung, die in Schritt A (iii) gebildet wurde, mit der entsprechenden Ableitung, die in Schritt B (iii) gebildet wurde.

2. Verfahren zum Identifizieren anomaler Spiegel eines extrinsischem oder intrinsischem Gerinnungsfaktors oder von Protein C, umfassend:
(i) Durchführen eines Gerinnungszeittests an einer Testprobe;
(ii) Messen des Grades der Gerinnselbildung als eine Funktion der Zeit für die Testprobe;
(iii) Bilden von sowohl der ersten als auch der zweiten Ableitungen der in Schritt (ii) gemessenen Funktion;
(iv) Bestimmen des Spiegels des Gerinnungsfaktors oder von Protein C aus einer Korrelation der ersten und zweiten Ableitungen.

3. Verfahren nach Anspruch 2, wobei der Spiegel des Gerinnungsfaktors oder von Protein C als Prozentsatz des Gerinnungsfaktors oder von Protein C ausgedrückt wird.

4. Verfahren nach Anspruch 2 oder Anspruch 3, wobei der Bestimmungsschritt (iv) umfaßt, daß man den Spiegel gegen die ersten und zweiten Ableitungen aufträgt.

5. Verfahren nach einem der Ansprüche 2 bis 5, wobei die Ergebnisse des Bestimmungsschritts als eine graphische Darstellung angegeben werden.

6. Verfahren nach Anspruch 4 oder Anspruch 5, wobei der anomale Spiegel aus der Analyse der Kurve oder graphischen Darstellung bestimmt wird.

7. Verfahren nach Anspruch 6, das darüber hinaus den Schritt umfaßt, daß mindestens eine Arzneidosis für einen Patienten oder ein klinischer Test, der an dem Patienten durchgeführt wird, aufgrund des Analyseergebnisses ausgewählt wird.

8. Verfahren nach einem der Ansprüche 1 bis 7, wobei der Gerinnungszeittest ein Prothrombinzeittest ist und das Verfahren zum Identifizieren extrinsischer Gerinnungsfaktorspiegel dient.

9. Verfahren nach einem der Ansprüche 1 bis 7, wobei der Gerinnungszeittest ein Test für die aktivierte partielle Thromboplastinzeit ist und daß das Verfahren zum Identifizieren intrinsischer Gerinnungsfaktorspiegel dient.

10. Verfahren nach Anspruch 8, das zum Bestimmen relativer Spiegel von extrinsischen Gerinnungsfaktoren II, VII und X zur Überwachung einer Cumarintherapie verwendet wird.

11. Verfahren nach Anspruch 9, das zum Bestimmen funktioneller Spiegel von intrinsischen Gerinnungsfaktoren XII, XI, IX, VIII, V, X und II zur Überwachung einer Heparintherapie dient.

12. Verfahren nach einem der Ansprüche 8 bis 11, wobei der Gerinnungszeittest unter Verwendung von Plasma durchgeführt wird, das einen Mangel an einem zu bestimmenden spezifischen Gerinnungsfaktor aufweist, gemischt mit entweder einer normalen Plasmaprobe oder einer Testprobe.

13. Verfahren nach einem der Ansprüche 1 bis 7, wobei der Gerinnungszeittest ein modifizierter Test für die aktivierte partielle Thromboplastinzeit ist und das Verfahren zum Identifizieren anomaler Protein C-Spiegel dient.

14. Verfahren nach Anspruch 13, wobei der Gerinnungszeittest durchgeführt wird, indem Protein C-Mangelplasma, gemischt mit entweder einer normalen Plasmaprobe oder einer Testprobe, verwendet wird.

15. Verfahren nach Anspruch 14, wobei der Gerinnungszeittest durch Aktivieren des Protein C mit einem Protein C-Aktivator durchgeführt wird.

16. Verfahren nach Anspruch 15, wobei der Protein C-Aktivator Schlangengift ist.

17. Verfahren nach einem der Ansprüche 1 bis 16, wobei der Grad der Gerinnselbildung durch Beobachten

des Grads der Trübung in der Probe gemessen wird.

18. Verfahren nach Anspruch 17, darüber hinaus umfassend die Schritte:
Bestimmen des Wertes der gesamten Trübungsänderung beim Gerinnungsendpunktmaximum in der normalen Plasmaprobe;
Bestimmen des Wertes der gesamten Trübungsänderung als dem Gerinnungsendpunktmaximum in der Plasmatestprobe; und
Vergleichen des Wertes der normalen Probe mit dem Wert der Testprobe.

19. Verfahren nach Anspruch 18, wobei der Wert der Testprobe zum Angeben der Fibrinogenspiegel verwendet wird.

Revendications

1. Procédé pour déterminer le niveau d'un facteur de coagulation extrinsèque ou intrinsèque ou de protéine C dans lequel:
(A)
(i) on effectue un test de temps de coagulation sur un échantillon de plasma normal,
(ii) on mesure le degré de formation de caillot en fonction du temps pour l'échantillon de plasma normal, et
(iii) on obtient la dérivée première ou seconde de la fonction mesurée dans l'étape (ii);
(B)
(i) on effectue le même test de temps de coagulation sur un échantillon de plasma expérimental,
(ii) on mesure le degré de formation de caillot en fonction du temps pour l'échantillon de plasma expérimental, et
(iii) on obtient la dérivée première ou seconde de la fonction mesurée dans l'étape (ii); et
(C) on compare la dérivée première ou seconde obtenue dans l'étape A (iii) avec la dérivée correspondante obtenue dans l'étape B (iii).

2. Procédé pour identifier des niveaux anormaux de facteur de coagulation extrinsèque ou intrinsèque ou de protéine C dans lequel:
(i) on effectue un test de temps de coagulation sur un échantillon expérimental;
(ii) on mesure le degré de formation de caillot en fonction du temps pour l'échantillon expérimental;
(iii) on obtient les dérivées première et seconde de la fonction mesurée dans l'étape (ii);
(iv) on détermine le niveau dudit facteur ou de protéine C à partir d'une corrélation desdites dérivées première et seconde.

3. Procédé de la revendication 2, dans lequel le niveau dudit facteur de coagulation ou de protéine C est exprimé en pourcentage dudit facteur de coagulation ou de protéine C.

4. Procédé de la revendication 2 ou de la revendication 3 dans lequel ladite étape de détermination (iv) comprend la construction de la courbe dudit niveau en fonction desdites dérivées seconde et première.

5. Procédé de l'une quelconque des revendications 2 à 4, dans lequel les résultats de l'étape de détermination sont fournis sous forme de représentation graphique.

6. Procédé de la revendication 4 ou de la revendication 5, dans lequel le niveau anormal est déterminé par analyse de la courbe ou de la représentation graphique.

7. Procédé de la revendication 6, comprenant en outre l'étape de choix d'au moins une dose de médicament pour un malade ou d'un test clinique à effectuer sur le malade d'après le résultat de l'analys .

8. Procédé de l'une quelconque d s revendications 1 à 7, dans l quel le test du temps de coagulation est un test de temps de prothrombin et le procédé vise à identifier les niveaux de facteur de coagulation extrinsèque.

9. Procédé de l'un quelconque des revendications 1 à 7, dans lequel le test du temps de coagulation est

un test de temps de thromboplastine partielle activée et le procédé vise à identifier les niveaux de facteur de coagulation intrinsèque.

10. Procédé de la revendication 8, qui est utilisé pour déterminer les niveaux relatifs des facteurs de coagulation extrinsèques II, VII et X pour contrôler le traitement à la coumarine.

11. Procédé de la revendication 9, qui est utilisé pour déterminer les niveaux fonctionnels des facteurs de coagulation intrinsèques XII, XI, IX, VIII, V, X et II pour contrôler le traitement à l'héparine.

12. Procédé de l'une quelconque des revendications 8 à 11, dans lequel ledit test de temps de coagulation est effectué en utilisant un plasma déficient en un facteur de coagulation spécifique à déterminer mélangé à un échantillon de plasma normal ou à un échantillon expérimental.

13. Procédé de l'une quelconque des revendications 1 à 7, dans lequel le test de temps de coagulation est un test de temps de thromboplastine partielle activée modifié et le procédé vise à identifier les niveaux anormaux de protéine C.

14. Procédé de la revendication 13, dans lequel ledit test de temps de coagulation est effectué en utilisant un plasma déficient en protéine C mélangé à un échantillon de plasma normal ou à un échantillon expérimental.

15. Procédé de la revendication 14, dans lequel le test de temps de coagulation est effectué en activant la protéine C avec un activateur de protéine C.

16. Procédé de la revendication 15, dans lequel l'activateur de protéine C est un venin de serpent.

17. Procédé de l'une quelconque des revendications 1 à 16, dans lequel on mesure le degré de formation de caillot en observant le degré de trouble dans l'échantillon.

18. Procédé de la revendication 17, incluant en outre les étapes de

détermination de la valeur de la modification de trouble totale au point final de coagulation maximum dans l'échantillon de plasma normal;

détermination de la valeur de la modification de trouble totale au point final de coagulation maximum dans l'échantillon de plasma expérimental; et

comparaison de ladite valeur de l'échantillon normal avec ladite valeur de l'échantillon expérimental.

19. Procédé de la revendication 18, dans lequel ladite valeur de l'échantillon expérimental est utilisée pour indiquer les niveaux de fibrinogène.

Figure 1

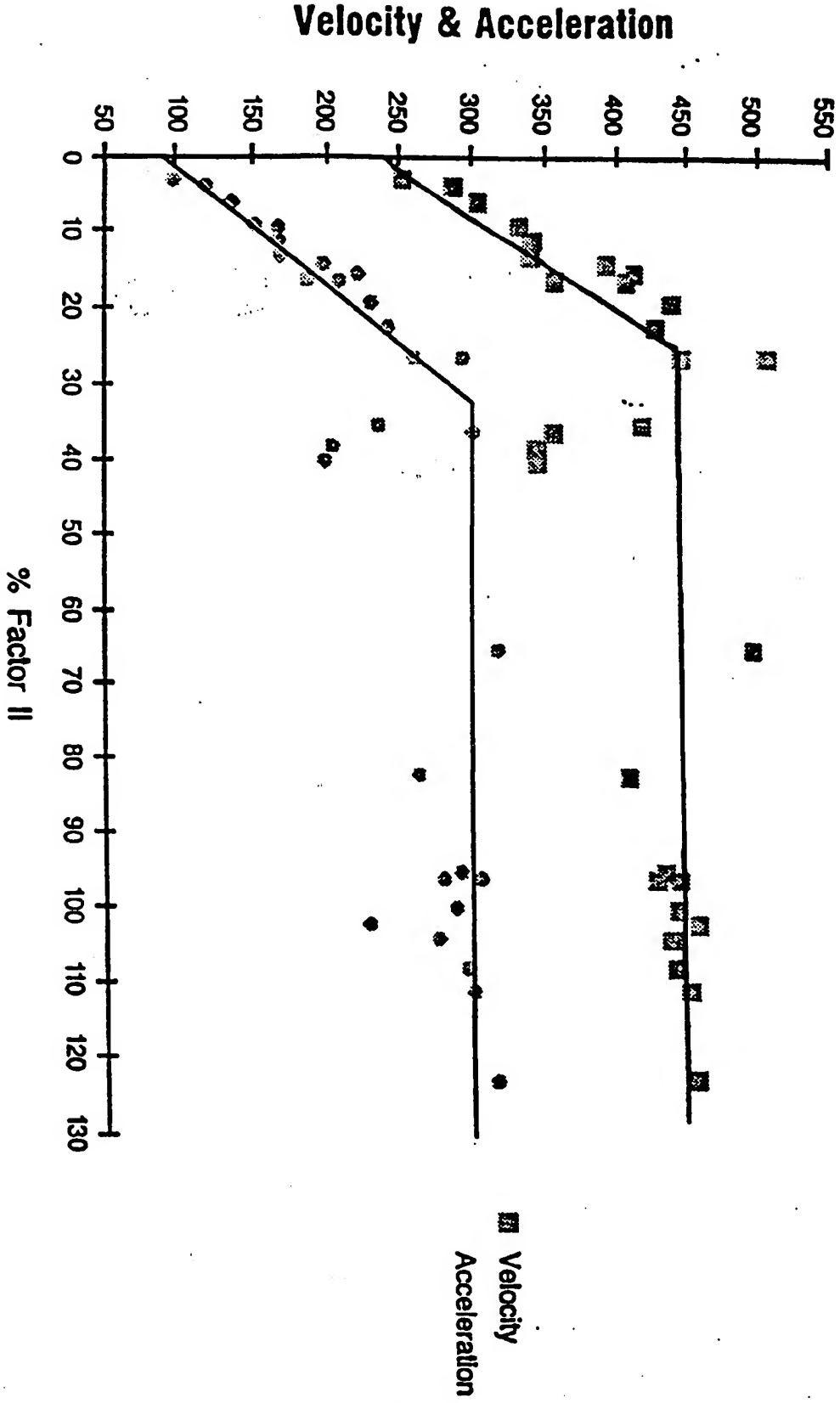
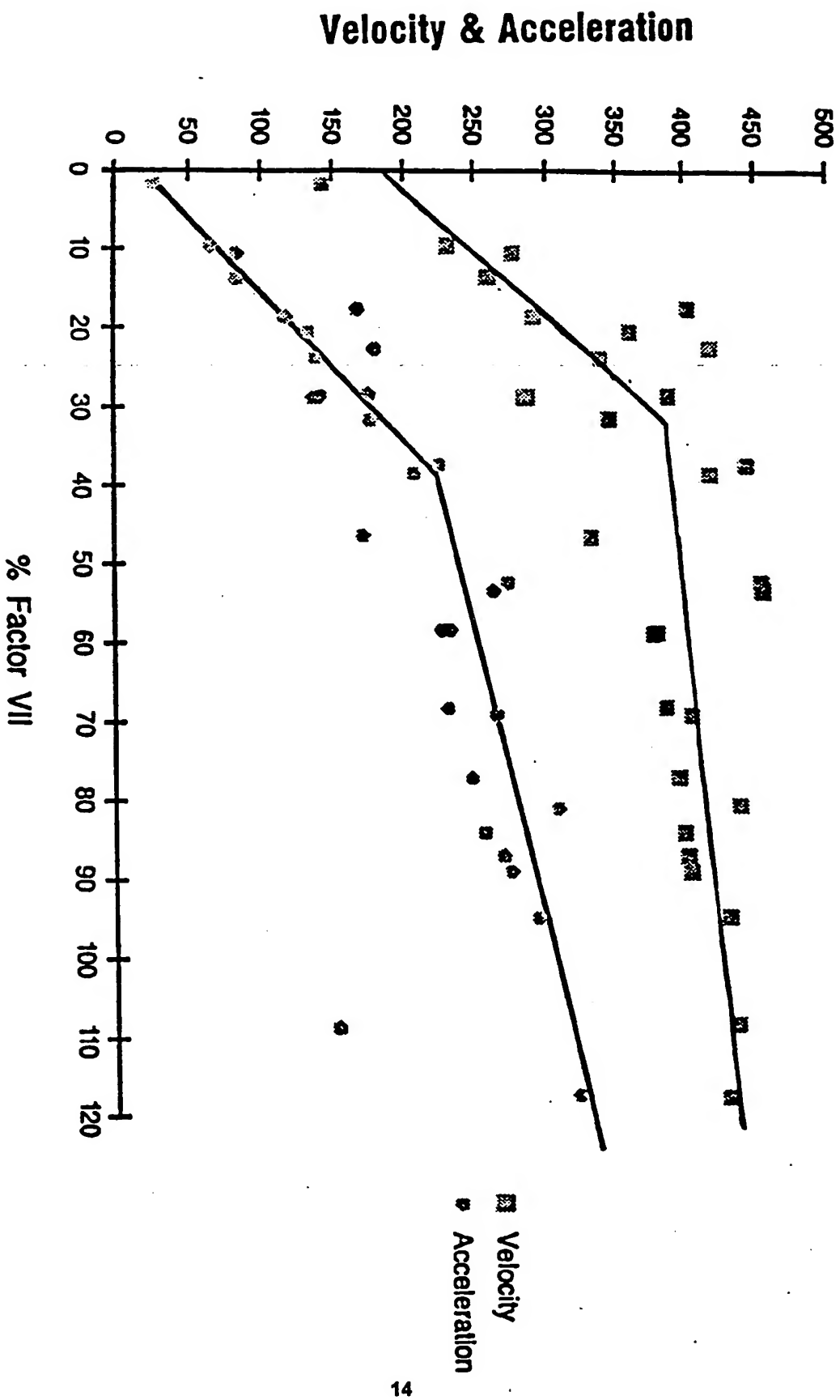

Velocity & Acceleration
50
100
150
200
250
300
350
400
450
500
550
0
10
20
30
40
50
60
70
80
90
100
110
120
130
% Factor II
Velocity
Acceleration

Figure 2


Velocity & Acceleration
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50
100
150
200
250
300
350
400
450
500
0
10
20
30
40
50
60
70
80
90
100
110
120
% Factor VII
Velocity
Acceleration

Figure 3

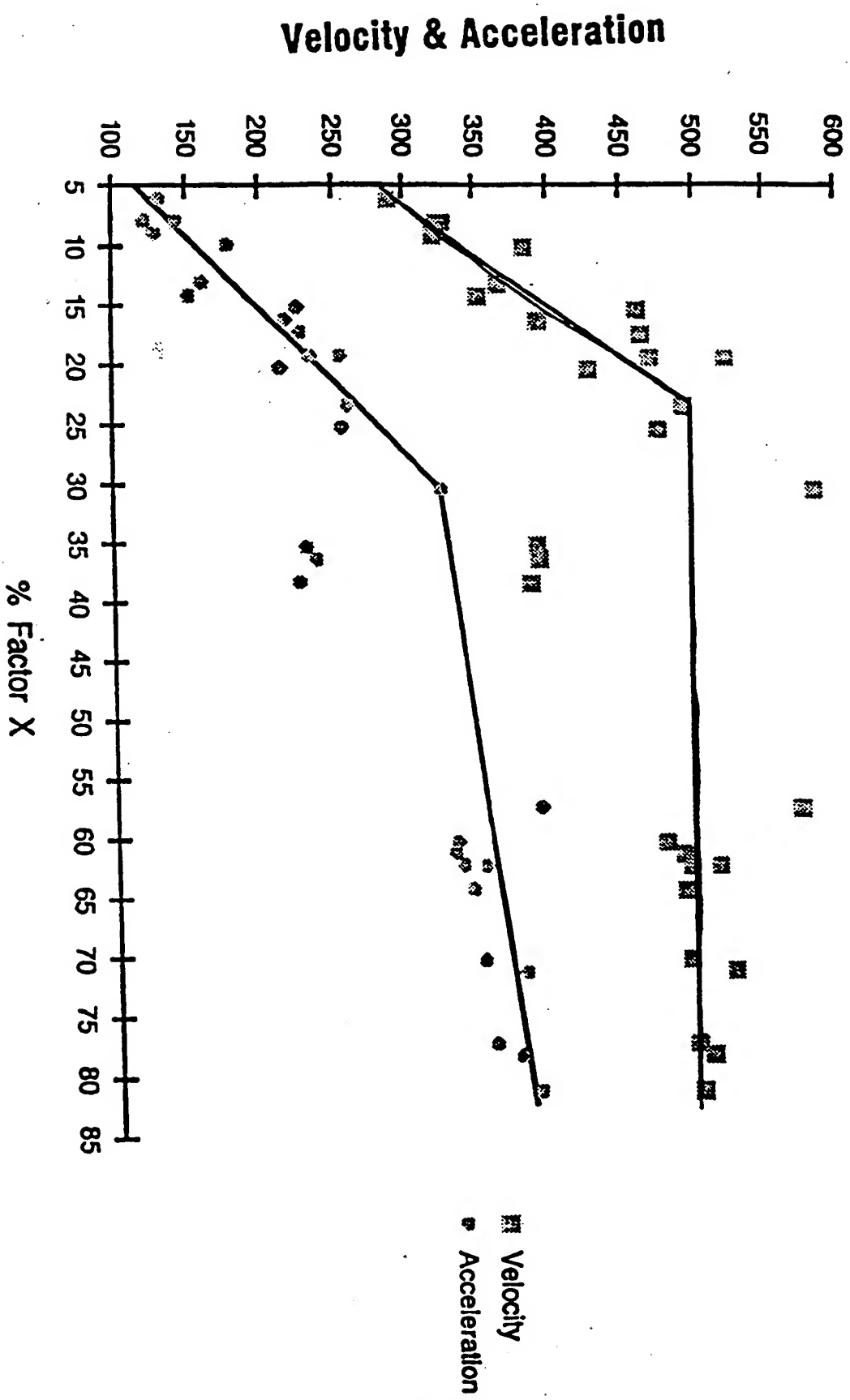

Velocity & Acceleration
100
150
200
250
300
350
400
450
500
550
600
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
% Factor X
Velocity
Acceleration

Figure 4

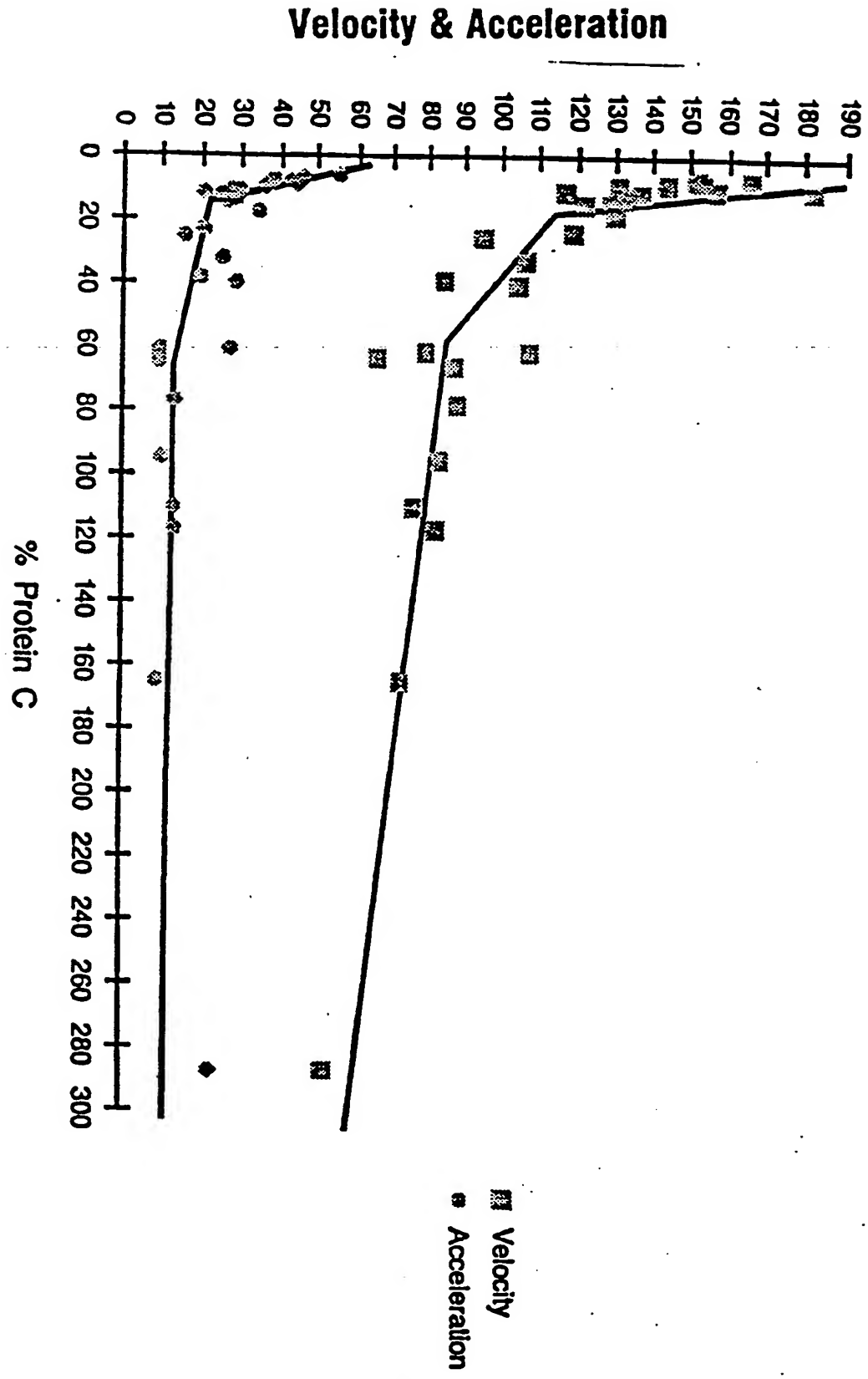
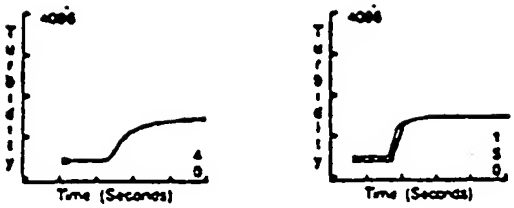
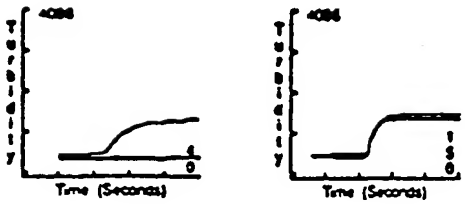

Velocity & Acceleration
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300
% Protein C
Velocity
Acceleration

Figure 5

PATIENT 1



PATIENT 2



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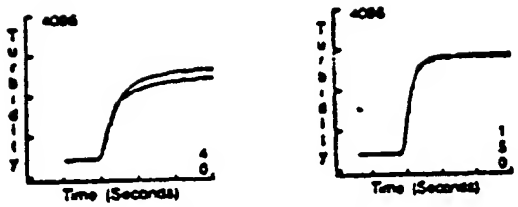

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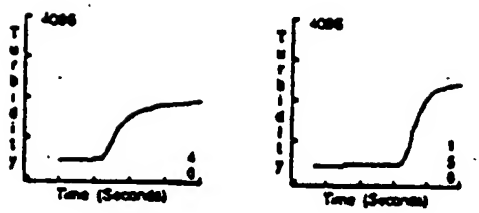


Figure 6

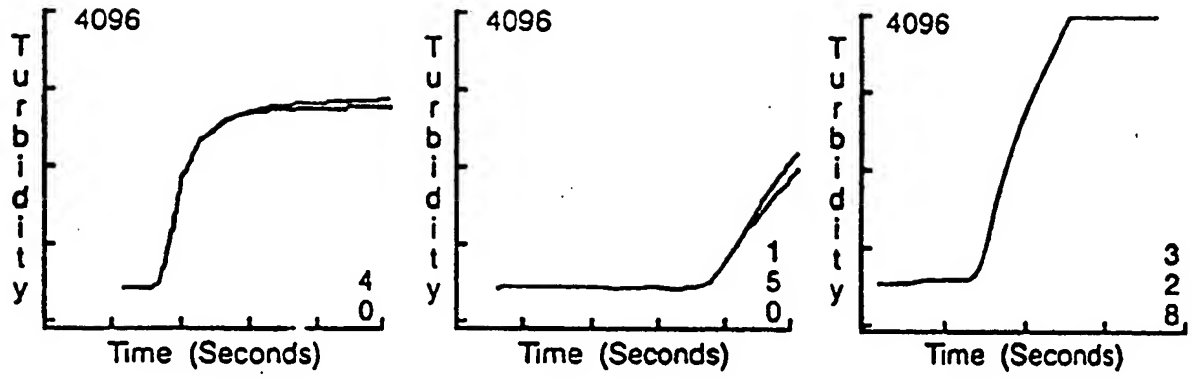

4096
Turbidity
40
Time (Seconds)
4096
Turbidity
150
Time (Seconds)
4096
Turbidity
328
Time (Seconds)

Figure 7

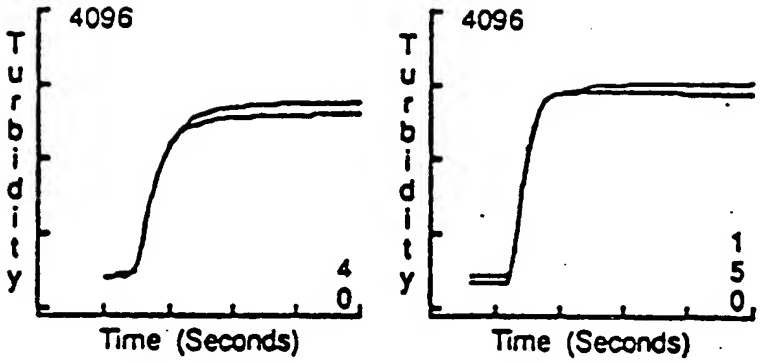
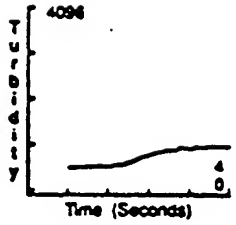
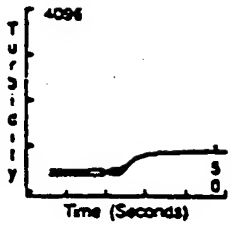

4096
Turbidity
40
Time (Seconds)
4096
Turbidity
150
Time (Seconds)

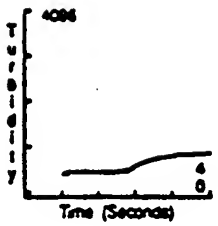
Figure 8

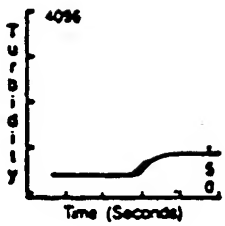
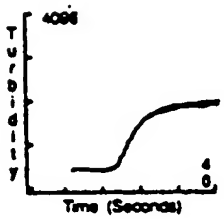
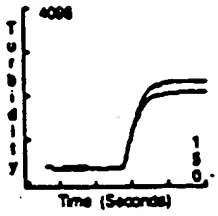
PATIENT 21





PATIENT 22





PATIENT 23